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What's Downstream? A Set of Classroom Exercises to Help Students Understand Recessive Epistasis †

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Undergraduate students in genetics and developmental biology courses often struggle with the concept of epistasis because they are unaware that the logic of gene interactions differs between enzymatic pathways and signaling pathways. If students try to develop and memorize a single simple rule for predicting epistatic relationships without taking into account the nature of the pathway under consideration, they can become confused by cases where the rule does not apply. To remedy this problem, we developed a short pre-/post-test, an in-class activity for small groups, and a series of clicker questions about recessive epistasis in the context of a signaling pathway that intersects with an enzymatic pathway. We also developed a series of homework problems that provide deliberate practice in applying concepts in epistasis to different pathways and experimental situations. Students show significant improvement from pretest to posttest, and perform well on homework and exam questions following this activity. Here we describe these materials, as well as the formative and summative assessment results from one group of students to show how the activities impact student learning.

INTRODUCTION

Interpreting the results of epistasis tests is an important learning objective in both genetics and developmental biology courses. In genetics courses, epistasis is typically taught as a means to describe an interaction between two genes in which the phenotype resulting from an allele of one gene "masks" the phenotype otherwise caused by alleles of the other gene. The mutation whose phenotype prevails is defined as epistatic to the other mutation. Epistatic relationships can be either recessive or dominant. When recessive epistasis occurs, the epistatic phenotype results from a homozygous recessive genotype. In contrast, dominant epistatsis occurs when only one copy of the epistatic allele is present.

One example of recessive epistasis described in genetics textbooks is flower color in the blue-eyed Mary plant (4). In this plant, two enzymes catalyze steps in the synthesis of blue pigment. If both Enzymes 1 and 2 are active, the plant will have blue flowers; if Enzyme 1 is active and Enzyme 2 is inactive, the plant will have pink flowers. However, if Enzyme 1 is inactive, the plant will have white flowers regardless of whether Enzyme 2 is active or inactive. Typically, students are asked to interpret results from genetic crosses among plants with different flower colors and they conclude that the mutation in the gene that encodes Enzyme 1 is epistatic to the mutation in the gene that encodes Enzyme 2. In addition, students are often asked to draw out an enzymatic pathway to describe this epistatic interaction. In this pathway, the gene that encodes Enzyme 1 is upstream of the gene that encodes Enzyme 2.

From examples such as flower color, students learn that a mutation in an upstream gene can mask another phenotype and often memorize the rule that the more upstream mutation is always epistatic. However, students are likely to encounter epistasis again in upper level courses where this rule does not always apply. For example, in developmental biology, students will likely study the genetics of a signaling pathway rather than an enzymatic pathway and can become confused when they learn that a mutation in an upstream gene is **not** the epistatic mutation. In fact, in signaling pathways, mutations in downstream genes are almost always epistatic (see (1) for more information on interpreting epistasis results with certain phenotypes).

In our combined experience teaching genetics and developmental biology courses for many years, we have repeatedly observed students trying to solve epistasis problems by applying a simplistic rule such as: "the upstream mutation is always epistatic" or memorizing specific pathways to help them answer questions. These strategies hinder conceptual understanding of the logic and the utility of epistasis testing,

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†Supplemental materials available at http://jmbe.asm.org

and they do not help students with application tasks such as analyzing the outcome of epistasis experiments or designing such an experiment to order steps in a pathway. To help teach the concept of recessive epistasis in the context of different pathway types, we have developed an in-class small group and clicker activity in which students analyze effects of mutations in a signaling and an enzymatic pathway that intersect. By combining the two pathways we encourage students to compare and contrast outcomes, which is a higher order cognitive skill (2).

Intended audience

The activity would be appropriate for any upper-level biology majors (including other more specific majors such as genetics, developmental biology, molecular biology) who have taken an introductory genetics course. Here, we present the results from the activity in an upper-level developmental biology course for undergraduate biology majors.

Learning time

The activity discussed here takes approximately 90 minutes. As shown in this activity, it was administered in one 75-minute class period, with an additional 15 minutes used at the beginning of the next class period for students to take the posttest and for instructors to discuss any outstanding difficulties. In a course with 50-minute class periods, the exercise could span two class periods, with the posttest at the end of the second class period. Follow-up homework and exam questions are also included.

Prerequisite student knowledge

In an upper-level developmental biology course, we assume that students have been exposed to Mendelian inheritance patterns and learned about epistasis in the context of enzymatic pathways in an introductory genetics course. The data described in this activity are from students who had taken introductory biology, genetics, molecular biology, and cell biology before enrolling in developmental biology, and thus had also studied the molecular nature of mutations and their possible impacts on an organism's phenotype, as well as the molecular components of many signaling pathways.

Learning objectives

Upon completion of this activity, students should be able to:

1. Deduce information about genes, alleles, and gene functions from analysis of genetic crosses.
2. Interpret the results of epistasis tests comparing the phenotypes that result from single mutations in two different genes with the phenotype of the double mutant.
3. Illustrate the interactions of genes that act in a pathway, based on their mutant phenotypes.

Approach

To help teach the concept of recessive epistasis in the context of different pathway types, we have used an in-class activity based on the pathway in Figure 1 where a regulatory and an enzymatic pathway are working together. Students are asked to predict the phenotypes of yeast with single and double loss of function (*lf*) mutations in the various pathway genes, and then use the phenotypic results to make conclusions about which mutation is epistatic. In order to reduce the cognitive load on students, we purposefully chose to make this example a hypothetical pathway with easy to understand yeast color phenotypes. However, to provide further deliberate practice, we also developed a set of homework problems and in-class formative assessments that require interpretation and design of epistasis experiments on signaling pathways in genetically tractable model organisms, such as *Caenorhabditis elegans* (*C. elegans).*

Students are first instructed to consider the hypothetical enzymatic pathway shown in Figure 1(A) and answer a series of questions about the color of the yeast if there were *lf* mutations in Genes 1 and/or 2 (see Appendix 1 for complete set of questions). In this pathway, cells growing in the presence of an artificial sugar called sucralose produce a green pigment. Recessive *lf* mutations in Genes 1 and/or 2 result in colonies that are either blue or colorless. For example, if yeast have a *lf* mutation in Gene 1 or *lf* mutations in both Genes 1 and 2, the colony will be colorless in both cases. This result occurs because if the production of upstream Enzyme 1 is blocked by mutation, the downstream intermediate Enzyme 2 also cannot be produced. In other words, if a yeast cell lacks Enzyme 1, it will be colorless regardless of whether Enzyme 2 is present. From this example, students can correctly infer that *lf* mutations in an upstream gene of an enzymatic pathway will generally be epistatic to mutations in a more downstream gene.

Students are then asked to consider the upper line of Figure 1(B) (blue). This hypothetical signaling pathway regulates the synthesis of Enzyme 2 in the enzymatic pathway according to the presence or absence of an artificial sugar called neotame as a ligand. Students then answer a series of questions about *lf* mutations of different combinations of genes in both the signaling and enzymatic pathways (see Appendix 1 for complete set of questions). In the signaling pathway, the large arrows represent not conversions of one intermediate to another, but rather regulatory steps, one negative (blunt arrow) and the rest positive (normal arrows), each controlling the activity or inactivity of the subsequent protein in the pathway. The output of the pathway depends on whether the transcription factor T is active or inactive, which in turn will determine whether

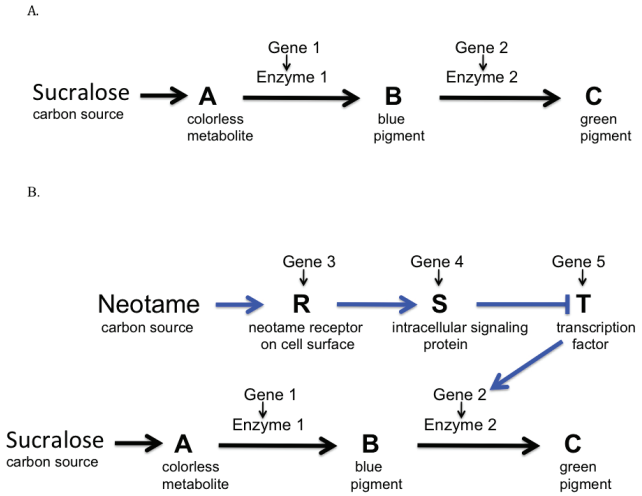

FIGURE 1. A hypothetical yeast enzymatic pathway regulated by a hypothetical signaling pathway. (A) Enzymatic pathway alone (see text for context). A, B, and C are small molecules. Heavy arrows represent enzymatic reactions, and light arrows represent expression of the genes that encode the corresponding enzymes in the pathway. This pathway is controlled by the presence or absence of sucralose. (B) The same enzymatic pathway (lower line), intersecting with a signaling pathway (blue line) that is controlled by the presence or absence of neotame. In the signaling pathway, R, S, and T are proteins with the functions shown, encoded by Genes 3, 4, and 5 respectively. Light arrows indicate gene expression, but here, heavy arrows represent regulatory interactions: pointed arrows indicate activation, and the blunt arrow indicates inhibition. For example, in the presence of both sucralose and neotame, if the receptor R is activated by neotame binding, it activates the protein S. If S is active, it inactivates the transcription factor T, which is otherwise active and is required to activate transcription of Gene 2.

Gene 2 is transcribed and thus, ultimately, the color phenotype of the yeast.

In this activity, students are encouraged to think of a signaling pathway as a series of on-off switches, each setting the state of the switch that follows it. For example, if Gene 5 has a *lf* mutation, then transcription factor T will not be made, Gene 2 will not be transcribed, and the yeast will be blue. Yeast with *lf* mutations in both Gene 4 and Gene 5 will also be blue, because the transcription factor T will not be produced when there is a *lf* mutation in Gene 5. In other words, if there is a *lf* mutation in Gene 5, the state of all switches upstream of Gene 5 is irrelevant, and the phenotype will be unaffected by additional upstream mutations. Thus, students can correctly conclude that *lf* mutations in a downstream gene of a signaling pathway will generally be epistatic to mutations in a more upstream gene.

In summary, in the pathway shown in Figure 1, a mutation in the upstream gene is epistatic in the enzymatic pathway (Fig. 1(A) and lower line of Fig. 1(B)), while a mutation in the downstream gene is epistatic in the signaling pathway (upper line of Fig. 1(B)).

PROCEDURE

Materials

This activity includes a paper handout (Appendix 1) and clicker questions (Figs. 2 and 3 and Appendix 2). If an instructor does not have access to clickers, the activity can be adapted as described further in Possible modifications. Appendix 2 includes all clicker questions as part of the PowerPoint for the class. Appendices 3 and 4 include homework and exam questions intended to be completed after this activity.

Student instructions

All instructions for students are included in the handout in Appendix 1; additional instructions that guide the students through the activity and questions are included on the PowerPoint slides that accompany the activity (Appendix 2).

Faculty instructions

Detailed directions of what faculty should do are provided below. The general schedule is: Day 1 – clicker pretest and small group activity; Day 2 – clicker posttest and assign homework. A PowerPoint file of the lecture slides including the pretest and posttest, and homework and exam questions (with and without answers) are included in Appendices 2–4. In addition, for a video of students in this course actively working on another similar activity and for advice on structuring group work, see http://www.cwsei.ubc.ca/resources/SEI_video.html.

Day 1 – Pretest. This activity begins with students answering five clicker pretest questions about epistasis (Fig. 2 and Appendix 2). In order for individual student thinking to be evaluated, the instructor tells the students to answer the questions without discussing them with their peers. Class results are not shared or discussed by the instructor; instead, students are told that the questions are intended to gauge what they currently knew about the topic, and that the answers will be revealed later.

Day 1 – In-class activity: yeast color. After the pretest, the instructor gives students a handout with the pathways shown in Figure 1(A) and (B) and a series of questions asking them to interpret the outcomes of mutations in two pathways that impact the yeast color (Appendix 1). The in-class activity is divided into two parts. The first part focuses on analyzing the effects of *lf* mutations in the enzymatic pathway (Fig. 1(A)). Students work in small groups to predict the color of the yeast colonies when a single gene has a *lf* mutation, and when two genes have *lf* mutations. The activity is divided into three parts and students are instructed to pause after they have completed each part for a whole class discussion. After students work on part one of the in-class

1. The synthetic pathway for the neurotransmitter norepinephrine involves two enzymes, tyrosine hydroxylase (TH) and DOPA decarboxylase (DOPA-D) and can be drawn as follows:

TH *DOPA-D*
Tyrosine → Dopamine → Norepinephrine

If you are analyzing the output of a cell that has a loss of function mutation (lf) in the DOPA-D gene and an lf mutation in the TH gene, which neurotransmitter will be produced?

a. Tyrosine
b. Dopamine
c. Norepinephrine

2. The synthetic pathway for the neurotransmitter norepinephrine involves two enzymes, tyrosine hydroxylase (TH) and DOPA decarboxylase (DOPA-D) and can be drawn as follows:

TH *DOPA-D*
Tyrosine → Dopamine → Norepinephrine

What is the epistatic relationship of the TH and DOPA-D genes?

a. Loss of function mutations in TH will be epistatic to loss of function mutations in DOPA-D
b. Loss of function mutations in DOPA-D will be epistatic to loss of function mutations in TH
c. You cannot determine the relationship from this pathway

3. Below is a signaling pathway. What will happen if there is a loss of function mutation in Gene 3?

Gene:	1 →	2 ⊣	3 ⊣	4 →	5 ⊣	6	Outcome:
Cell X	on	on	off	on	on	off	"A" fate
Cell Y	off	off	on	off	off	on	"B" fate

Answer	Cell X will be	Cell Y will be
A.	"A" fate	"B" fate
B.	"A" fate	"A" fate
C.	"B" fate	"B" fate
D.	"B" fate	"A" fate
E.	Not enough information	Not enough information

4. Below is a signaling pathway.
What will happen if there is a loss of function mutation in both Gene 3 and Gene 5?

Gene:	1 →	2 ⊣	3 ⊣	4 →	5 ⊣	6	Outcome:
Cell X	on	on	off	on	on	off	"A" fate
Cell Y	off	off	on	off	off	on	"B" fate

Answer	Cell X will be	Cell Y will be
A.	"A" fate	"B" fate
B.	"A" fate	"A" fate
C.	"B" fate	"B" fate
D.	"B" fate	"A" fate
E.	Not enough information	Not enough information

5. You are studying programmed cell death in *C. elegans* and have isolated two genes *ced-3* and *ced-4*, which have the following phenotypes when mutant:
(Loss of function = *lf*; Gain of function = *gf)*
ced-4 (lf): no cell death
ced-3 (lf): no cell death
ced-3 (gf): excessive cell death

You make a strain of worms that is mutant for both *ced-4 (lf)* and *ced-3 (gf)*. The phenotype is excessive cell death. You can conclude:

a. *ced-4 (lf)* is epistatic to *ced-3 (gf)*
b. *ced-3 (gf)* is epistatic to *ced-4 (lf)*
c. You cannot make a conclusion based on this information.

FIGURE 2. Epistasis pretest and posttest questions. Correct answers are underlined.

activity (Appendix 1, questions 1–3), the instructor brings the entire class together and asks two clicker questions: 1) a repeated question from the handout which asks students to predict the yeast color if there are *lf* mutations in both Gene 1 and Gene 2 (Fig. 3 and Appendix 1, question 3), and 2) follow-up question 1 which focuses on determining the epistatic relationships between mutations in the two genes in the pathway (Fig. 3). Students are encouraged to discuss the answers with their peers and, after clicker votes are recorded, the instructor discusses the answers to these questions with the whole class.

The instructor then asks students to work in groups on the second part of the activity, which includes consideration of the signaling pathway in addition to the enzymatic pathway (Fig. 1(B)). As before, students work in groups to answer the questions on the handout (Appendix 1), which ask them to predict the output of the enzymatic pathway in the presence of *lf* mutations in different genes of the signaling pathway. The instructor then re-asks three questions from the handout as clicker questions (Fig. 3 and Appendix 1, questions 6, 8, and 12). The activity concludes with follow-up clicker question 2, in which students draw a conclusion about the epistatic relationships between mutations in the three genes in the signaling pathway (Fig. 3). Students are encouraged to talk about the clicker questions with their peers and, after clicker votes are recorded, the instructor discusses the answers to these questions with the whole class.

After students complete the activity and clicker questions, the instructor uses the pathway in Figure 1 to contrast the behavior of gene products in signaling vs. regulatory

Question from handout asked as clicker question	% correct
3. In a double-mutant strain with *lf* mutations in both Gene 1 and Gene 2, the colonies that form on sucralose plates will be a. colorless (white). b. blue. c. green.	98
Follow-up question 1: For the simple metabolic pathway you just looked at, a) *lf* mutations in Gene 1 will be epistatic to mutations in Gene 2. b) *lf* mutations in Gene 2 will be epistatic to mutations in Gene 1. c) neither of these mutations will be epistatic to the other.	90
6. In a strain that has an *lf* mutation in Gene 4, the colonies that form on sucralose and neotame plates will be a. colorless (white). b. blue. c. green.	90
8. In a strain that has an *lf* mutation in Gene 4, the colonies that form on sucralose plates will be a. colorless (white). b. blue. c. green.	95
12. In a double-mutant strain that has *lf* mutations in Genes 3 and 5, the colonies that form on sucralose and neotame plates will be a. colorless (white). b. blue. c. green.	100
Follow-up question 2: For the signaling pathway shown, *lf* mutations in Gene 5 will be a) epistatic to *lf* mutations in Gene 3, but not in Gene 4. b) epistatic to *lf* mutations in Gene 4, but not in Gene 3. c) epistatic to *lf* mutations in Genes 3 and 4.	80

FIGURE 3. Clicker questions from in-class activity and follow-up. Correct answers are underlined and percentages of correct responses from students in the developmental biology class described in the discussion are shown.

pathways (Appendix 2, slides 19 and 20). The instructor then asks students: "How would you order two genes when *lf* mutations in each produce identical phenotypes, as is the case with *lf* mutations in in either Genes 3 or 4?" (slide 21). One solution is to search for a gain of function (*gf*) mutation in one of the genes and then make a double mutant. Discussing this issue allows for a review of *gf* and *lf* mutations, and their utility in epistasis testing (slide 22). Finally, the instructor shows students the pathway from the handout again, and reminds them of the behavior of gene products with an inhibitory (negative) regulatory role, such as Protein S in Figure 1(B) (slide 23). A common problem for students is interpreting the negative regulation incorrectly, not realizing that if Protein S is inactivated by mutation, the inhibition of Protein T will be removed, Protein T will be active, and the yeast will be green. It is important to discuss this potential difficulty before moving on to the application questions.

Day 1 – In-class activity: application questions using *C. elegans*. Next, students are asked to apply what they have learned to an authentic research scenario where two different single-gene *lf* mutations in *C. elegans* result in opposite phenotypes with respect to the vulva (the egg laying organ): multivulva vs. vulvaless (5, 6). The instructor presents a slide with information about the genes involved in this pathway, and the phenotypes that result from mutations in these genes (Fig. 4 and Appendix 2, slide 25). In brief, cells can contribute to the vulva by taking on either a primary or secondary fate; cells that become tertiary contribute to the surrounding epidermis. When too many cells take on primary or secondary fates, multiple vulvae form (multivulva). When no cells take on primary or secondary fates, no vulva can form (vulvaless). Students then work in groups to draw possible pathway diagrams indicating how the protein products of these two genes could interact in a regulatory pathway to determine normal vulval development (Fig. 4(A) and Appendix 2, slide 26). There are two possible ways to order the regulatory steps that are consistent with the phenotypes of single mutations in these genes. The instructor does not yet discuss the correct possible pathways, but instead, next shows a slide with a new piece of information about the phenotype of the double mutant; namely, the phenotype of the double mutant is the same as the phenotype of one of the single mutants, *lin 1* (Fig. 4(B) and Appendix 2, slide 27). This new information limits the answer to one pathway order and the students are asked to use clickers to individually choose the correct pathway. Students then discuss this question with their peers and vote again, followed by a whole class discussion, in which the instructor can ask individual students to explain how they deciphered the possible pathways and came up with their answer. There are several problems that students often reveal, which can be explored by the instructor at this point. One problem is that students forget that the pathway shows the normal function of each gene; if they think they are indicating the outcome when a gene's function has been lost, they draw the pathway incorrectly. Another problem is the correct use of inhibitory arrows: for example, if a gene's normal role was to inhibit the next gene in the pathway, then if the first gene is nonfunctional, the second gene will now be active, rather than inhibited. Helping students step through correctly illustrating and interpreting such pathways allows them to correctly determine the outcome of an epistatic relationship (learning objective 2) and depict gene interactions in a pathway (learning objective 3).

Day 2 – Posttest. At the beginning of the next class period, students are asked to answer the pretest questions again as a posttest (Fig. 2 and Appendix 2, slides 28–32). For these questions, students vote individually, and peer discussion followed by a re-vote only occurs if fewer than 75% of the students answer correctly. The histograms of student answers are only shown to the class after the re-vote to prevent any bias from seeing the distribution of other students' answers (5).

Suggestions for determining student learning

Formative assessment questions on epistasis. In addition to the in-class clicker questions, students complete homework questions following the in-class activity and posttest on the topic of epistasis using *C. elegans* mutant phenotypes as the context (see Appendix 3 for homework questions that are linked to the learning objectives). The questions involve using the mutant phenotypes, information about the nature of the mutations, and epistasis tests to either determine the correct order of genes in a signaling pathway, or to construct a pathway.

Summative assessment of student learning. On the unit exam (two weeks after the activity) and on the final exam, students answer several questions related to determining the order of genes in a signaling pathway using information from epistasis tests (see Appendix 4 for exam questions that are linked to the learning objectives). For example, on the final exam, students are asked to recognize which of four possible signaling pathways are consistent with data they are given about mutant phenotypes, and then to pick and explain which of the pathways is correct after they know the outcome of an epistasis test.

Sample data

Students are primarily assessed through multiple-choice clicker questions, and a combination of multiple-choice and short-answer homework and exam questions. Results are shown in Figures 3, 4, and 5.

Safety issues

There are no safety issues associated with these activities.

A. Preliminary question for group discussion

If the genes shown below interact with each other in the same signaling pathway to help determine which cells take on vulval fate, and the end outcome of your pathway is "vulval fate", what are all the possible ways you could draw these two genes interacting in a pathway?

let-60(lf): no cells (among the vulval precursor cells) take on vulval fates (Vul phenotype).
lin-1(lf): all cells (among the vulval precursor cells take on vulval fates (Muv phenotype).

Answer: there are two possible ways to draw the pathway with this information:
lin-1 —| *let-60* → vulval fate
let-60 —| *lin-1* —| vulval fate

B. Additional evidence and clicker question

You know the following additional information about mutants in the pathway of genes that help to specify the vulva:

Mutant gene	Phenotype
let-60(lf)	Vul; all precursor cells make epidermis (3° fate).
lin-1(lf)	Muv; all precursor cells adopt 1° or 2° vulval fate.
let-60(lf);lin-1(lf)	Muv; all precursor cells adopt 1° or 2° vulval fate.

Of the pathways shown below, which is/are consistent with the above data? (Pathways show each gene's normal function.)

	Individual Vote	Revote After Peer Discussion
a) *lin-1* —| *let-60* → vulval fate	18%	5%
b) *lin-1* → *let-60* —| vulval fate	3%	0%
c) *let-60* —| *lin-1* —| vulval fate	20%	90%
d) *let-60* → *lin-1* —| vulval fate	5%	0%
e) (a) and (c)	55%	5%

FIGURE 4. In-class application questions. (A) This question asks students to draw possible pathways of gene interaction, given the phenotype of *C. elegans* mutants. (B) Clicker question in which students must choose the correct pathway based on additional evidence presented. Student performance on this question is shown for the individual vote, and following peer discussion. Correct answers are underlined.

DISCUSSION

Field testing and evidence

The data presented in this paper are from students who took a junior/senior Developmental Biology course, taught by Jennifer Knight, at the University of Colorado in 2011 (demographics shown in Table 1). This series of activities was used in two previous years as well, with similar results.

TABLE 1.
Student demographic information.

Category	Demographic Information
Class Standing	16% junior, 82% senior
Ethnicity	33% Non-Caucasian
Sex	53% Female
Major	82% Molecular, Cellular, and Developmental Biology, 11% Biochemistry, 7% Other

Student performance on clicker questions from the in-class activity. Between 80% and 100% of the students answered the clicker questions from the handout and the follow-up questions correctly (Fig. 3), suggesting that the activity is accessible to students, and helps them learn about the concepts of epistasis. However, students initially struggled to apply the concept of epistasis to a real research scenario involving *C. elegans* (Fig. 4). When students voted individually on a question about determining the order of genes in a pathway, their scores were low, indicating difficulty with interpreting the outcome of inhibitory arrows in the pathway, and with integrating information about the double-mutant phenotype. Encouragingly, after students were able to talk about the questions with their peers, 90% of the students selected the correct answer (Fig. 4), supporting previous work indicating the value of peer discussion (3, 6, 7). Thus, by the end of the class period, the activity was successful in helping students apply the appropriate logic in epistasis problems involving different kinds of pathways

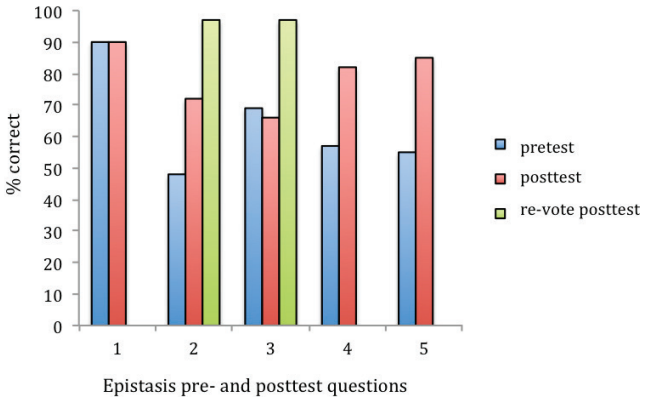

FIGURE 5. Performance (% correct) on the pretest and posttest questions. Pretest performance is represented by the blue bars and posttest performance by the red bars. For two posttest questions (2 and 3), students discussed their answer and re-voted (green bars). Average performance on the posttest, both before and after re-vote, is significantly higher than on the pretest (paired *t*-test, $p < 0.05$).

(enzymatic and signaling), correctly interpret signaling pathways with inhibitory interactions, and predict the epistatic mutant gene in either type of pathway.

Measuring initial conceptual difficulties with an in-class pretest and re-testing after instruction with a posttest. The pre-/posttest questions ask students to: predict the phenotype when there are two recessive mutations in an enzymatic pathway (question 1), determine which of two mutant genes in the given pathway is epistatic (question 2), predict the phenotype when there are one or more recessive mutations in a signaling pathway (questions 3 and 4), and determine which of two mutant genes in the signaling pathway is epistatic (question 5) (Fig. 2). The average pretest score was 61.2% and the results separated by question (Fig. 5) suggest that students are more familiar with the analysis of mutations in an enzymatic pathway than in a signaling pathway (question 1 vs. questions 3 and 4), and that they have difficulty establishing epistatic relationships in both situations (questions 2 and 5). The identical questions were posed to students as a posttest two days later to determine if the activity improved student understanding of epistatic relationships in both enzymatic and signaling pathways. The average posttest score on the individually answered questions is 78.9%, a significant improvement from the pretest (paired *t*-test, $p < 0.05$); furthermore, Figure 5 shows that there was an improved understanding of epistasis on several individual questions. On the two most challenging posttest questions (questions 2 and 3), students first answered the question individually and then the instructor asked the students to discuss the question and vote again. Students did not see the graph of student votes or have any other instructor input. After this re-vote, the average score on questions 2 and 3 increased to 90%, suggesting that the students can interpret and answer questions about epistasis, but continue to benefit from discussing their ideas with their peers. Student learning from peer discussion has been documented in other courses that involve clickers (3, 6, 7). Student understanding of epistasis is further demonstrated by their performance on homework and exam questions given after this activity.

Homework and exam questions. Student homework and exam questions are included in the Appendix. For the homework, students were allowed to work with peers and/or the instructors, so scores are generally >90% correct. On the final exam, students achieved 93% correct on question 1, and 87% correct on question 2. These data suggest that repeated practice in solving problems involving recessive epistasis results in improved learning by the end of the course.

Possible modifications

For faculty members who do not have access to clickers or do not use them regularly, students can hold up colored cards with the answer choices or can answer all the questions on paper. For the in-class clicker questions, the instructor can have students work in groups, and call on individual groups to explain their answer to the class.

SUPPLEMENTAL MATERIALS

Appendix 1: In-class epistasis activity. Correct answers are included at the end of the activity.

Appendix 2 and 2A: PowerPoint file of class materials. Appendix 2 contains all slides for conducting class, including the pre- and posttest and in-class clicker questions. Appendix 2A is the instructor version, including answers and explanations to all questions.

Appendix 3: Homework questions. Sample homework questions are provided, aligned to each of the learning goals. Correct answers are included, following the questions.

Appendix 4: Unit exam and final exam questions. A sample of unit and final exam questions are shown, aligned to each of the learning goals. Correct answers are included, following the questions.

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responses (exempt status, Protocol No. 0108.9) was granted by the Institutional Review Board, University of Colorado, Boulder.

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